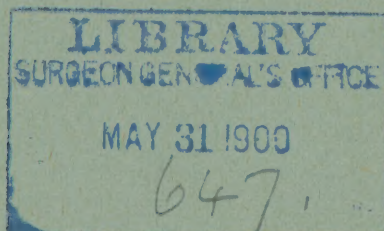


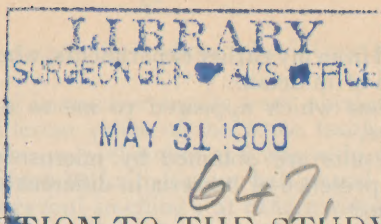
MILLER (W.D.)

An Introduction to the Study of the Bacterio-Pathology of the Dental Pulp.

BY W. D. MILLER, M.D., D.D.S., BERLIN.

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AN INTRODUCTION TO THE STUDY OF THE BACTERIO-PATHOLOGY OF THE DENTAL PULP.

By W. D. MILLER, M.D., D.D.S., BERLIN.

AFTER the decay of the teeth, the diseases of the pulp, particularly where their results, such as pericementitis, periostitis, alveolar abscess, etc., are taken into consideration, form the most important of the pathological processes with which we as dentists have to do.

The investigations of the last ten or fifteen years have furnished a satisfactory explanation of the chief phenomena associated with the decay of the teeth, but we are still very much in the dark as to the bacteriological aspect of the diseases of the pulp, pericementum, etc.

We assume, in a general way, that bacteria must in some manner be connected with these processes; but the vital questions as to what bacteria we have to do with, how they obtain access to the pulp, what toxic products they form, etc., have not been solved.

In the experiments recorded in the following pages, I have endeavored to furnish an introduction to the study of the bacterio-pathology of the pulp, and hope to have laid a foundation upon which future experimenters may build, in order to arrive eventually at results not only of scientific value, but which may be applied in the daily treatment of diseased conditions of the teeth and neighboring parts.

These investigations were begun in the spring of 1891, and had made considerable progress when an attack of influenza compelled me to give up work for three months, during which time all of my cultures died, so that in the fall of 1892 I was forced practically to begin my experiments anew. A similar mishap occurred again in the summer of 1893, when many of the newly started cultures, although transplanted weekly during my absence, again died out, while others became impure.

Only now, since I have for the third time occupied myself uninterruptedly for nearly a year, many hours daily, with this question, am I able to communicate the results of my experiments.

I have, in all, examined over two hundred and fifty tooth-pulps, in one direction or another, but shall present only a few of the general

results derived from my earlier experiments, whereas the last fifty pulps will be treated of in detail.

The questions which appeared to me to demand solution in the first place were,—

1. What results are obtained by microscopical investigations in regard to the presence of bacteria in different diseased conditions of the dental pulp?
2. What micro-organisms are obtained by the method of pure culture?
3. What effect has the incorporation of pure cultures from diseased pulps on the animal body?
4. What effect has the incorporation of diseased pulps, or pieces of such, on the animal body?
5. How are the intense putrefactive processes in the pulp to be accounted for, and what significance have they?
6. Have we remedies at our disposal which are capable of rendering putrid pulps innocuous for the animal organism?

Microscopical Examination of Diseased Pulps.

The teeth to be examined should be thoroughly washed, dried, and split open by the excising forceps, in such a manner that no possible contamination of the pulp from without may take place. By means of a platinum needle, a minute quantity of serum, pus, or decomposed pulp-tissue is spread out upon a cover-glass and stained in the usual manner for bacteriological preparations. The stains used were chiefly methylen-blue, methyl-violet, Ribbert's solution of dahlia, and carbol-fuchsin. Methylen-blue is a weak stain, but has the advantage of coloring the tissues but faintly, compared to the bacteria. The other solutions, particularly carbol-fuchsin, are much more powerful, but they stain the pus and blood-corpuscles, fibrin, etc., so intensely that the bacteria may be lost to sight.

On the other hand, bacteria which take on the color with difficulty, and which might be easily overlooked when treated with methylen-blue, often appear quite distinct when fuchsin is used. Both solutions, then, have their advantages and disadvantages.

When the pulp-chamber is open, all possible species of bacteria that occur in the mouth may, of course, be brought into contact with the pulps. I have therefore considered such cases but little in my investigations, and turned my attention almost exclusively to cases where the pulp-chamber was still closed and the pulp not contaminated by material from without. In some instances, however, where there was a small opening into the pulp-chamber, I took the material for examination from some point far removed from the exposure; for instance, when there was a slight opening on the mesial surface of a molar, the material for examination was taken from the distal root.

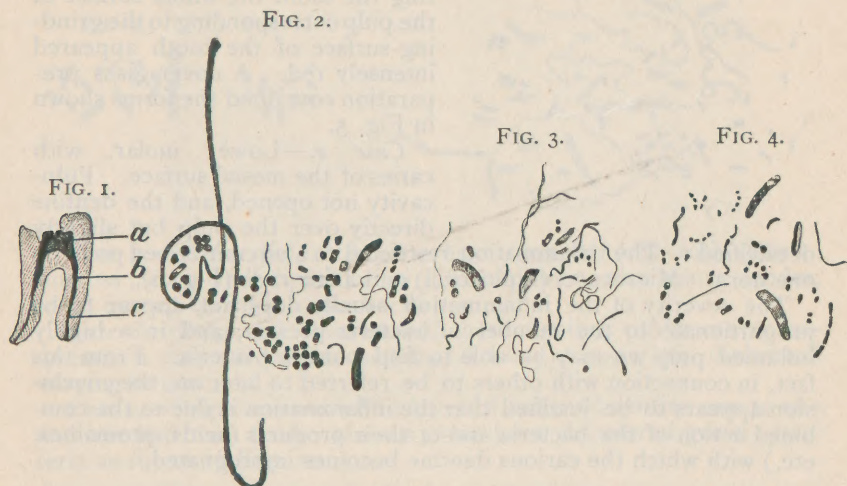
In a few cases, however, an examination was made where the pulp-chamber was completely open and filled with food. These examinations presented some points of interest which will be briefly noticed.

Examination of Teeth with Open Pulp-Chamber.

In three cases where the pulp-chamber was wide open and filled with food and débris, I found among different species a great pre-

ponderance of large, round cocci, whose physiology I have not yet studied more closely.

Very interesting and instructive results were obtained by examining material from different parts of the same tooth. In the case illustrated in Fig. 1, the pulp-chamber at *a* was wide open and filled with food particles, which had a half-sour, half-putrid odor; at *b* the pulp was putrid and very foul-smelling; at *c* there was a small abscess, filled with pure white pus, while the tissue between this point and the apex of the root was highly inflamed and bright red.



Material from the pulp-chamber (*a*, Fig. 1) contained the forms shown in Fig. 2; material from point *b* those shown in Fig. 3, and from the point *c* those shown in Fig. 4. We perceive a gradual diminution of the large cocci, and the appearance of small, delicate cocci and diplococci.

Examination of the Inflamed Pulp.

Cases of incipient inflammation of the pulp are difficult to obtain, because usually the teeth are not extracted until the pain has become very severe, and on splitting open the tooth we generally find suppuration already present. Even in cases where the inflammation appears to be in its beginning, the microscope will often reveal the presence of pus. Consequently I have had occasion to examine only a comparatively small number of cases of inflammation of the pulp in its earliest stage. *A priori* we should expect the bacteria which occur in the earliest stages of inflammation to be met with later on in suppuration and gangrene of the pulp, because it is not probable that one species causes the inflammation and then makes room for another species to attend to the suppuration. At the same time, in these later processes we should expect to find a much larger number of bacteria as well as a greater number of different kinds; particularly where a considerable period of time has elapsed between the appearance of the inflammation and the consequent suppuration, there is a possi-

bility or even probability that in the mean time new species will have been added from the carious dentine, while those already present will have greatly multiplied.

Out of a series of cases the two following may serve to present the bacteriological aspect of an inflamed pulp :

Case 1.—Lower molar, extensive caries on the grinding-surface. Pulp covered with a thick layer of partially decalcified dentine ; the softened dentine at the time highly discolored and fairly dry. On splitting the tooth the whole surface of the pulp corresponding to the grinding-surface of the tooth appeared intensely red. A cover-glass preparation contained the forms shown in Fig. 5.

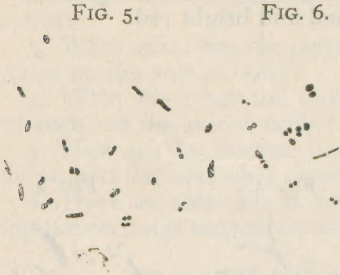


FIG. 5.

FIG. 6.

Case 2.—Lower molar, with caries of the mesial surface. Pulp-cavity not opened, and the dentine directly over the pulp but slightly decalcified. The inflammation restricted to a circumscribed point on one horn. Micrococci (diplococci) and a few rods (Fig. 6).

The severity of the inflammation usually does not appear to be proportionate to the number of bacteria present, and in a highly inflamed pulp we may be able to find but few bacteria. From this fact, in connection with others to be referred to later on, the conclusion appears to be justified that the inflammation is due to the combined action of the bacteria and of their products (acids, ptomaines, etc.) with which the carious dentine becomes impregnated.

Microscopical Examination of Purulent Pulps.

I have made no attempt in my experiments to arrive at any possible distinctions which might be supposed to exist between the bacteria associated with different conditions of suppuration of the pulp ; for while it is not permissible *a priori* to exclude the possibility of different forms of suppuration of the pulp—abscess, ulcer, local or total suppurative gangrene—being brought about by specifically different kinds of bacteria, it is exceedingly improbable, and experiments thus far made point to an opposite conclusion.

Case 1.—Pulps covered with an extremely thin layer of semi-decalcified dentine, highly inflamed ; on the surface a thin film of pus. Bacteria present shown in Fig. 7.

Case 2.—Softening of the dentine up to the pulp, but the latter still covered on all sides ; superficial suppuration. Examination of the pus showed cocci and bacilli.

Case 3.—Pulp not exposed ; small abscess in one horn ; plump cocci and diplococci ; a few isolated bacilli (Fig. 8).

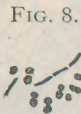
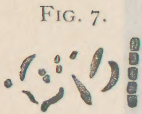
Case 4.—Molar. Pulp on one of the mesial horns covered with but a very thin layer of decomposed dentine ; in the crown of the pulp an immense abscess with yellowish-gray pus ; numerous cocci and diplococci ; a few thick bacilli and some thin, pointed bacilli ; a few vibrios ; many long spirochætes (Fig. 9).

Case 5.—Pulp covered by a layer of softened dentine one and one-half to two mm. thick. The whole pulp-chamber and a large part

of the root-canals filled by an immense abscess ; numerous cocci and diplococci ; several fine vibriones and long, thin threads.

Case 6.—Molar. Pulp covered with softened dentine ; slight superficial suppuration of one of the mesial horns ; masses of long spirochaetes in almost pure cultures ; a few vibriones and very large bacilli (Fig. 10).

FIG. 9.



Case 7.—Molar pulp. At one horn covered only with débris ; abscess ; cocci and diplococci of different sizes ; also many slender, long, curved bacilli (comma bacilli).

Case 8.—Pulp still covered with hard dentine. Abscess, consequently a case of pulpitis septica (Arkövy) ; cocci and diplococci varying in size.

Case 9.—Pulps covered with a layer of softened dentine one to one and one-half mm. thick ; abscess ; one horn discolored green ; bacteria as shown in Fig. 11.

FIG. 10.



An almost pure culture of *Spirochaete dentium* from a suppurating pulp. After the photograph. 1000:1.

Case 10.—Thick layer of softened dentine. Directly over the pulp a layer of hard dentine as thick as writing-paper ; fine open abscess with pure white pus and very red line of demarcation ; bacteria as seen in Fig. 12.

Case 11.—Pulp covered with layer of softened dentine two mm. thick. Superficial suppuration of one horn, which, as well as the underlying dentine, is discolored green ; cocci and diplococci.

Case 12.—Similar to 11, but without the green discoloration. Bacteria as in Fig. 13.

Case 13.—Similar to Case 11. Large abscess with viscid pus ; only isolated plump diplococci of different sizes.

Case 14.—Similar to Case 11. Abscess with yellowish-white pus ; bacteria as in Fig. 14.

FIG. 11.



Cover-glass preparation from an abscess of the pulp. Pus-corpuscles, micrococci, bacilli, and leptothrix. After the photogram. 1000:1.

Case 15.—Pulp covered with a very thin layer of dentine. Suppurating, putrid (gangrenous) ; various forms of bacteria ; comma bacilli and spirochaetes particularly numerous ; also large, snake-shaped cells (Fig. 15).

FIG. 12.

FIG. 13.

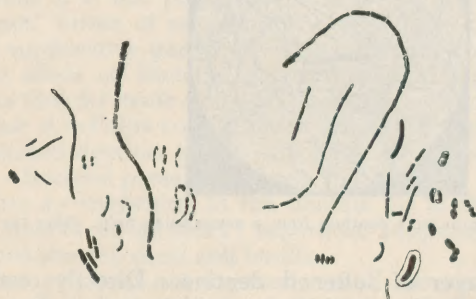
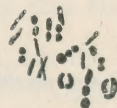


FIG. 14.



A further series of cases yielded the same results, from which it may be seen that we almost without exception have to do with mixed infections. The repeated appearance of spirochaetes and vibrios (comma bacilli) is rather surprising. We had an almost pure culture of the former in Case 6 (Fig. 10). The small cocci and diplococci are to be considered as a constant occurrence ; they almost always appear separately, not in chains ; even *short* chains are only occa-

sionally found. The number of cocci present varies considerably. Sometimes only isolated cells, in other cases large masses are to be found, but rarely in such quantities seen in Fig. 16.

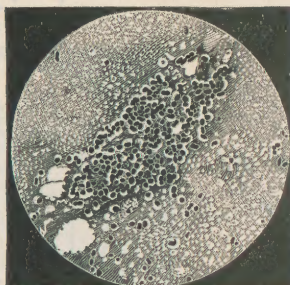
Microscopical Examination of Putrid Pulps.

Case 1.—Bicuspid. Gutta-percha filling over an exposed pulp, the latter totally putrid; very foul-smelling bacteria, as in Fig. 17. Only

FIG. 15.



FIG. 16.



Masses of cocci from an abscess of the pulp. After the photogram. 1000:1.

three colonies developed in the culture, two of small cocci and one of larger, oblong cocci.

Case 2.—Pulp covered with softened dentine one-half mm. thick; pulp totally putrid. Bacteria as in Fig. 18. Only one species of cocci developed in pure culture.

Case 3.—Molar with acute caries on grinding-surface; pulp completely covered; putrid, foul-smelling. Masses of cocci and bacilli, the latter partly club-shaped (Fig. 19).

Case 4.—Pulp covered with only a thin layer of decomposed den-

FIG. 17.

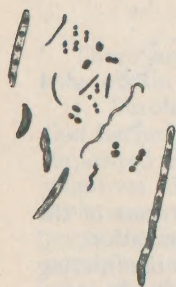
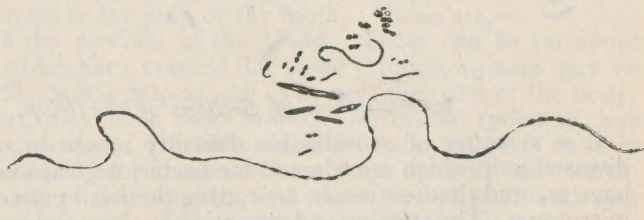


FIG. 18.



tine; pericementum much thickened; alveolar abscess; pulp black, foul-smelling. Bacteria as in Fig. 20.

Case 5.—Pulp covered with softened dentine; purulent, putrid (gangrenous). Bacilli and long threads with spores (Fig. 21).

Cases 6 and 7 gave the forms seen in Figs. 22 and 23.

Other cases yielded similar results.

We see that the small cocci and diplococci occurring in all the

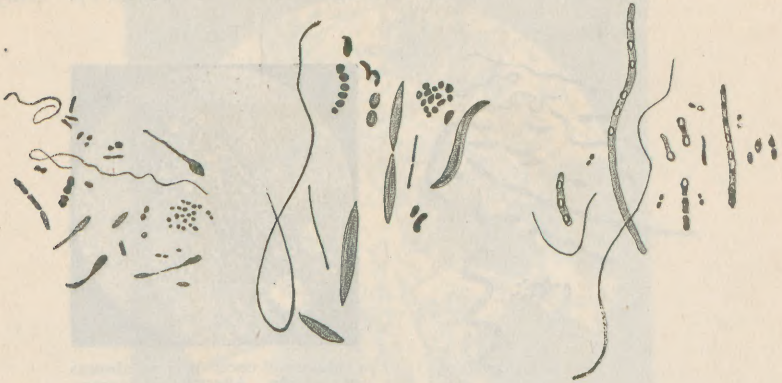
cases of suppuration of the pulp are also to be found in putrid pulps. But other forms seem to have been added which are not met with so often in the processes of suppuration, especially the long, stiff, pointed bacilli and threads.

In these cases very instructive results are also obtained by examin-

FIG. 19.

FIG. 20.

FIG. 21.



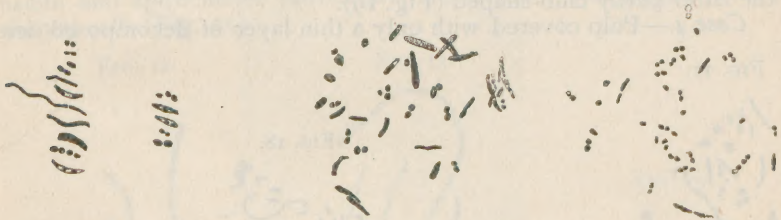
ing material from different parts of the pulp, especially where, as is a common occurrence, the crown is in a putrid condition, while suppurating processes are still going on in the root. Figs. 24 and 25 illustrate a case of this nature. The first is from a preparation obtained from the coronal portion of the pulp which was intensely putrid; the second from the radical portion, which was alive and suppurating.

FIG. 22.

FIG. 23.

FIG. 24.

FIG. 25.



Examination of Sections of the Pulp.

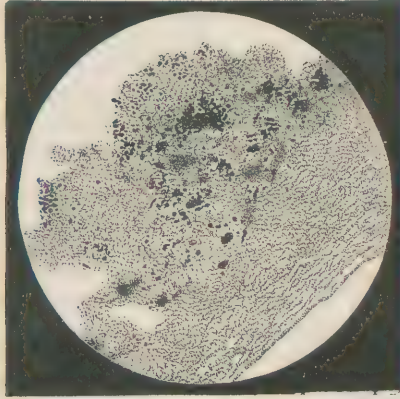
It is a matter of considerable difficulty to obtain sections of the diseased pulp which are adapted for bacteriological examination. I have secured the best results by cutting the fresh pulp on the freezing microtome. The staining and mounting is also accompanied by great difficulties, since the thin, delicate sections, when placed in alcohol, shrink to about half their size, become shriveled and turned up at the edges; in the further treatment they also often get into folds or tear, so that they become utterly unfit for examination.

I have succeeded best by taking each section separately upon the end of a glass rod and bringing it successively into twenty per cent., forty per cent., seventy per cent., and finally into absolute alcohol.

The staining (after Gram), clearing up, etc., are also done on the rod. With care and experience we succeed in this way in obtaining specimens which show no folds and but slight shrinkage.

The number of pulps that I have examined in this way has been too limited to enable me to form a conclusion regarding the very important question as to how the bacteria spread in the tissue of the pulp. In the accompanying illustration (Fig. 26), taken from the margin of a large ulcer of the pulp, we see a gradual invasion of the tissue by micrococci, singly or in masses.

FIG. 26.



Invasion of the pulp-tissue by micrococci, from margin of an ulcer. After the photogram. 400: 1.

In sections I have found a preponderance of micrococci, singly or in groups, less often in short chains. (Consult in this connection the monograph of Rothman, "Patho-Histologie der Zahnpulpa und der Wurzelhaut," Budapest, 1889.)

I. REGARDING THE MANNER IN WHICH THE INFECTION OF THE PULP IS BROUGHT ABOUT.

There are various channels by which it is supposed that bacteria may obtain access to the pulp of the tooth. These are,—

1. Through the medium of the blood. There can be no doubt that bacteria which have entered the blood through wounds may be deposited in the dental pulp as well as in any other part of the body, wherever there may be a *locus minoris resistentiæ* (point of less resistance) at the time. This manner of infection suggests itself in particular in connection with the necrotic pulp, but we have unfortunately no means of determining, with certainty, whether and with what frequency infections of this nature actually take place.

2. It has been suggested that bacteria may work their way to the pulp through the thin layer of cementum and the dentine at the neck of the tooth. It is, however, rather doubtful whether they can traverse the cementum and very fine ramifications of the dentinal tubules, unless caries is present. This point might and should be determined by a microscopical examination of an adequate number of sections from the neck of the tooth.

3. The chief channel of infection is through the decayed dentine. This point might almost be taken for granted, but it may be of interest to note that in a case of suppuration of the pulp, sections made of the overlying dentine showed the same forms of bacteria as were found in the pulp itself.

The irritation of the pulp brought about by the products of decomposition in the carious dentine render it more susceptible to the action of the bacteria themselves. The question whether an infection of the pulp may take place as long as it is covered by hard dentine, must be answered in a positive sense. Out of fifty cases of diseased pulps, I noted three in which the pulp was still covered by a layer of hard dentine about one-eighth to one-quarter mm. in thickness.

Culture Experiments : Methods and Results.

The teeth employed were almost exclusively such as had been extracted at the Dental Institute of Berlin between the hours of eleven and one, and were kept in a moist condition till I was able to examine them, which was usually between three and four o'clock.

They were first brushed in a five per cent. solution of sublimate, and then transferred to absolute alcohol for a short time, whereupon they were separately taken out with sterile pliers and the alcohol burned off. Care must be taken that the tooth does not become so hot as to kill the bacteria contained in the pulp. The object of this treatment is to devitalize all the bacteria upon the surface of the tooth, so that the pulp may not by any possibility become contaminated by them. The tooth is then split open with sterilized excising forceps, which must be so applied as not to come into contact with the pulp when the tooth splits, the purpose of this precaution also being to get at the pulp without its becoming infected from without. Having in this manner laid open the pulp-chamber, the material obtained is made use of for the customary cultures and infections.

The cultures were made on glycerin-agar in test-tubes and in Petri vessels, also on gelatin.

Where there was sufficient material at my disposal, small pieces were introduced under the skin of mice, or the pulp was rubbed up in sterile water and the emulsion thus obtained injected either into the abdominal cavity or subcutaneously. Subcutaneous inoculations of this kind usually led to the formation of abscesses, the pus of which was used for further culture experiments.

Finally, a mouse was inoculated from each agar culture when two or three days old, for the purpose of determining whether pathogenic bacteria were present or not.

Of the general results obtained, I give the following :

Of eighty-five cases of suppuration of the pulp, the cultures yielded in thirty-three, only cocci ; in twenty-five, only bacilli ; in nineteen, different forms ; while in nine cases no growth took place.

Cultures from fourteen gangrenous pulps yielded, in seven, only cocci ; in two, only bacilli ; in four, cocci and bacilli. In one case no growth was obtained.

Out of forty cases of putrid pulps, I found, in seventeen, only cocci ; in six, only bacilli ; in nine, cocci and bacilli, and in eight no growth occurred.

Out of seventeen cases of inflamed pulps I found, in four, only

cocci ; in three, only bacilli ; in five, cocci and bacilli ; and in five no growth took place.

It must strike every one that the results of the culture experiments do not tally with those of the microscopical examination. While a careful microscopical examination of the diseased pulp almost invariably revealed a mixed infection, the pure cultures show, in the majority of cases, either only cocci or only bacilli. This occurrence is, however, not inexplicable, because if one species is in strong preponderance it may easily, especially if it proliferates quickly, overrun and cover up the others.

It also happens that where one species is but scantily represented, it may, for some reason or other, not develop at all upon the agar plate, although it may belong to the cultivable species.

A third possibility to be considered in the putrid pulps is that the bacteria visible under the microscope may already have died from lack of food. Besides, in the fourth place, many species of bacteria occurring in the diseased pulp, vibriones, spirochaetes, the stiff pointed bacilli and threads, have not been found cultivable on artificial media anyway ; and possibly there are still other uncultivable pulp-bacteria.

In detailing the results of the culture experiments, I will restrict myself to the last fifty cases examined. These include nine cases of inflammation of the pulp, twenty-three of suppuration, sixteen of putrefaction, and two of alveolar abscess.

Of the nine inflamed pulps, two developed each two species of cocci ; two developed only one species each ; one gave both cocci and bacilli, and one only bacilli. In three cases the culture remained sterile.

Of the twenty-three suppurating pulps, six gave only one species of cocci ; three gave two species each ; two gave one species of bacilli ; in two other cases, two species of bacilli developed ; in five, one species of cocci and one of bacilli ; in two, two species of cocci and two of bacilli ; in one case, three species of cocci and one of bacilli ; in two cases no growth occurred.

Of the sixteen putrid pulps, seven developed each one kind of coccus ; in two cases, two species of cocci appeared ; in one, three species ; in another, one species of bacilli ; in one case, two kinds of bacilli ; in two cases, one species of bacilli and one of cocci ; in two cases, no growth. From the pus of one of the alveolar abscesses I cultivated two species of cocci ; from that of the other, only one species of cocci.

In twenty-seven of the fifty cases the material for examination was planted directly on gelatin as well as on glycerin-agar. Growth took place in nineteen cases ; in eight the gelatin remained sterile ; in three it became strongly liquefied ; in one case only slightly so.

In a later series of nine diseased pulps, growth on gelatin occurred seven times.

It could not be ascertained from these gelatin cultures whether only one or more species had developed in each test-tube, but the cultures certainly prove that species of bacteria that grow on gelatin occur with considerable constancy in the diseased pulp.

The following very incomplete grouping of the bacteria of the pulp may serve our purpose until further experiments enable us to make a better one :

Bacteria of Pulp	Cultivable	Bacilli.		
		Micrococci	Growing on gelatin	Liquefying.
	Non- cultivable		Not growing on gelatin.	Non-liquefying.
		Stiff, pointed bacilli.		
		Vibriones (comma bacilli).		
		Spirochaetes.		
		?	?	?

I. THE CULTIVABLE BACILLI OF THE DENTAL PULP.

Among the fifty cases under consideration there were, as stated above, six cases where no growth occurred, consequently forty-four in which colonies developed. In nineteen of these forty-four, colonies of bacilli developed in the glycerin-agar cultures; in fifteen cases one species, and in three two species.

The supposition would seem justifiable that species of bacilli occur in the pulps which are not cultivable on glycerin-agar, since the microscopical examination almost invariably reveals bacilli, while in the culture media they appear in only thirty-six per cent. of all the cases.

I attribute comparatively little importance to the cultivable species of bacilli found in the diseased pulps as causes of inflammation and suppuration, since I did not meet with any species which appeared

FIG. 27.



FIG. 28.

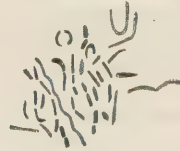


FIG. 29.



with any considerable degree of constancy, and with few exceptions inoculations with the pure cultures resulted negatively. I shall therefore not devote much time to the bacilli.

Among the different species of bacilli the form represented in Fig. 27 was met with most frequently. It grows as bluish-gray colonies, which, under a power of seventy-five diameters, show long loops of bacilli, and otherwise present much similarity to colonies of anthrax.

A second species occurs in the form shown in Fig. 28. It grows on glycerin-agar in grayish-blue, moist-looking colonies, which appear yellowish under the microscope, and have an indented, rather transparent margin. The larger colonies often show a streaked or veined appearance.

A third species produces small gray colonies (under the microscope, iron-gray), which show long loops of bacilli under a power of seventy-five diameters. Short, thick bacilli, single or in chains (Fig. 29).

I have, further, twice met the form given in Fig. 30, once as a pure culture from an inflamed pulp.

The species of bacilli reproduced in Fig. 31, which forms pointed, often more or less curved, bacilli, I have only met in my former cultures. Lately I have not been able to find it.

The three following species, which were met with each one in over two hundred cases, seem to me to be deserving of particular notice :

1. An upper lateral incisor, which had been treated eighteen months previously, presented an attack of acute pericementitis which made it necessary to remove the filling. When the cotton with which the root-canal had been filled was taken out, it was followed by a flow of pus, out of which I was able to cultivate a short bacillus that proved to be highly pathogenic for mice and rabbits.

A blood-serum culture applied interperitoneally to mice caused death in thirty hours. Masses of short, thick bacilli in the peritoneal exudations, also a few rods in the blood.

This bacterium grows on gelatin, liquefying it, and is probably identical with *Bacterium gingivæ pyogenes*, formerly described by me.

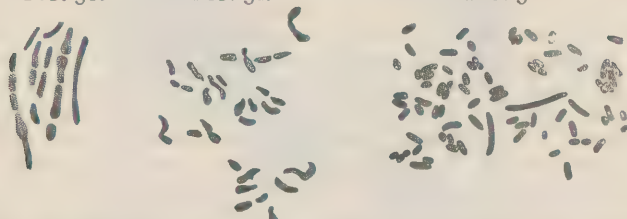
2. A left upper molar had been filled directly over a freshly devitalized pulp. It had remained without reaction for two years. Suddenly pericementitis of a very violent nature set in, which necessitated the extraction of the tooth.

I found the pericementum very much thickened and infiltrated with pus, and the pulp in a semi-liquid, exceedingly putrid condition. In the latter a bacillus was found which caused death of mice by blood-poisoning inside of twenty-six hours.

FIG. 30.

FIG. 31.

FIG. 32.



A cover-glass preparation of this micro-organism is represented in Fig. 32, after the photogram, 1000 : 1.

3. Incisor, with a large, ill-fitting cement filling. The pulp underneath not quite exposed, but in a purulent condition, *without smell*. The culture revealed very large, rapidly growing, blue-gray colonies, that may attain a diameter of five mm. in twenty-four hours. Short bacilli with rounded ends 0.1 ccm. of a bouillon culture one day old, or 0.1 of the condensation liquid of an agar culture, injected subcutaneously or into the peritoneal cavity of mice, caused death within thirty hours. Numerous bacilli in the blood.

It is very striking that these three cases, out of more than two hundred, should all have occurred on teeth which had formerly been filled. It would be an important question, which I have not as yet had time to investigate, how these three species of bacilli would affect other animal organisms, and whether they might have played a rôle in the various cases of death resulting from dental diseases which are from time to time reported.

2. THE MICROCOCCI OF THE DENTAL PULP.

a. Growing on Gelatin.

Whether a bacterium grows on gelatin or not, depends upon the composition of the latter and upon the temperature at which the cultures are kept.

Furthermore, many bacteria seem to possess the power of adapting themselves to artificial culture-media. In fact, nearly all of my cultures seem to grow better on gelatin now than they did formerly, and many which at first showed no growth gave very good cultures on gelatin after having been cultivated for a number of generations on glycerin-agar. It may be that the gelatin now in use has a slightly different composition or reaction from that formerly employed, but undoubtedly the chief reason for the better growth of the bacteria is their power of accommodation.

The growth of micro-organisms upon gelatin depends, accordingly, upon the composition and reaction of the gelatin, upon the temperature at which the cultures are kept, and finally upon the circumstance whether the bacterium has been previously cultivated upon other media or not.

I made use of the beef-water-peptone gelatin, containing 0.5 per cent. of common salt and ten per cent. of gelatin. It was kept at a temperature ranging from 21° to 23° C.

a. Micrococci which Liquefy the Gelatin.

I obtained micrococci that liquefy the gelatin from four different pulps: Nos. 16, 30, 31, and 49. Further examination of the cultures showed in Cases 16 and 30 *Staphylococcus pyogenes albus*; in Cases 31 and 49 *Staphylococcus pyogenes aureus*. No. 31 was the case of an incisor which had been treated six years previously and the root-canal filled with iodoform cotton. There was still a strong smell of iodoform. The tooth had been perfectly quiet for six years. An attack of dull pain in the evening caused the patient to consult me on the following morning, when I found an accumulation of pus about the apex of the root. The culture yielded *Staphylococcus pyogenes aureus* and another species of cocci to be described below. In Case 49 there was a profuse accumulation of pus about the root of an upper incisor, with considerable œdema of the lips. The culture showed the presence of *Staphylococcus pyogenes aureus* alone.

β. Non-liquefying Species of Micrococci.

Among the species of micrococci that grow upon gelatin without liquefying it, I mention as the first, but by no means most important: *Streptococcus pyogenes*. In the fifty cases under consideration there grew only four times a coccus that developed cultures which appeared in every way so similar to cultures of undoubted *Streptococcus pyogenes*, planted at the same time, that I felt justified in pronouncing them identical.

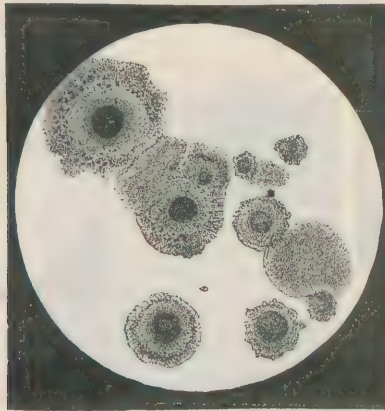
The chief importance is to be attached to a species of micrococci, or to a group of nearly allied species frequently met with in diseased pulps. I have, however, not as yet been able to determine whether we here have to do with one species or with several nearly allied species.

It is very well known to every bacteriologist how difficult it may be to establish the identity or non-identity of two species of micrococci. Even the question as to whether *Streptococcus pyogenes* and *Streptococcus erysipclatis* are identical is still differently answered by different authorities, although it has been a subject of constant investigation for the last ten years.

It will therefore be readily understood that when it comes to settling the relationship between several species, any one who is not able to devote his whole time to the work will find it simply impossible to arrive at a definite conclusion. Consequently, it must be left for future investigation to determine whether the forms now to be described belong to one species or to a group of nearly allied species.

In Figs. 33 and 34 I have reproduced two of the different forms in which cultures on glycerin-agar appear. Line cultures on the surface of the agar appear after one to two days as if strewn over with quite small, very bright glass beads, intermixed with larger, flat gray or bluish-gray colonies. The bead-like colonies scarcely attain a diameter of one mm.; the flat colonies are often two or more mm. in diameter. In some cultures only, or almost only, the former kind of colonies develops; in others we may find only the latter. It is sometimes impossible to determine that the flat colonies lie more superficially than the bead-like colonies. I think I may take for granted

FIG. 33.



Pure culture of micrococci from the pulp on glycerin-agar. 35:1.

that the evaporation of the condensation liquid on the surface of the agar covers the young colonies with a thin layer of albumen, which offers great resistance to the spreading of the colonies on the surface.

The small colonies are often hard or cartilaginous; on trying to lift them with the platinum needle they slip along before the point. Under the microscope they appear very opaque, nearly black, while the flat colonies appear thin and colorless at the margin, with a round or star-shaped nucleus in the center; or the whole surface, near the middle, appears as if covered with particles of dust. Sometimes again these particles appear only toward the margin (Fig. 33). They show a more compact nucleus, and a border of a darker color with a greenish tinge. The border, which is highly refractive, is either continuous or consists of a number of nodes which possess a radiated structure. Where it is not present, the margin of the colony is very uneven. In other cultures the bead-like colonies are quite missing; we see only flat, bluish-gray colonies that appear colorless under the microscope or faintly yellow toward the center. On gelatin this

species grows in the shape of bright dots or of very thin blue colonies, that appear colorless under the microscope, with an uneven margin.

A second form of growth is seen in Fig. 34. Round, bluish to gray colonies, which reveal under the microscope a dense, dark, round center with a thin margin of various width. In the very large colonies the nucleus may be missing. We meet with various modifications of this nuclear form of colonies, while in older cultures, where the colonies do not lie closely together, very graceful and pretty shapes appear (Fig. 35).

The form of growth most frequently observed shows the following characteristics:

The colonies grow rapidly on agar, and attain their height in three days, when they may reach two mm. in diameter. *The young colonies are often intensely blue, later they become bluish gray to gray, but do not, in this respect, always act alike.* The large ones frequently possess a whitish center and a transparent rim.

FIG. 34.

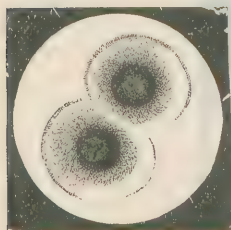


FIG. 35.

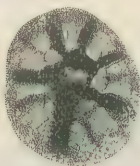
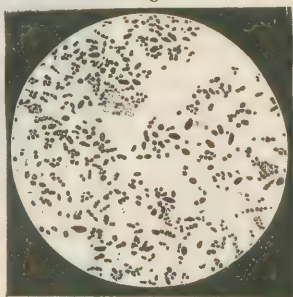


FIG. 36.



Cover-glass preparation of micrococci from the pulp. After the photograph. 1000:1.

Under the microscope the colonies look gray or colorless; sometimes the center is of a yellowish tinge. The surface appears partly homogeneous, partly as if strewn with few to very many dust-particles; margin smooth, or furnished with very flat indentations or short loops.

In Figs. 36-39 we have the appearance of a cover-glass preparation under one thousand diameters; small cocci and diplococci, almost always singly, only exceptionally in short chains of two to six links, with numerous large olive-shaped involution forms.

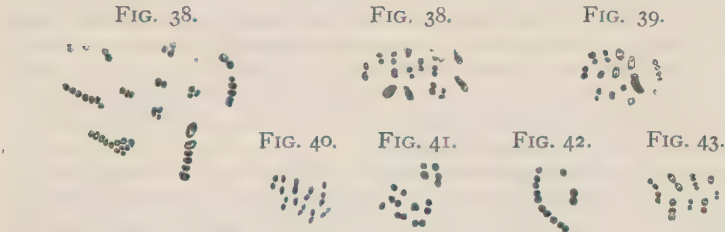
These are the morphological characteristics that we meet with repeatedly in the groups of cocci which we are at present considering. Sometimes the cocci may appear a little larger, another time a little plumper. Other small variations may also occur (Figs. 40-43); but these differences are not such as could not be easily explained by some slight change in the conditions of growth.

Sometimes, without apparent reason, the involution forms are so large and numerous that the cocci are forced quite into the background (Figs. 44-47).

Cover-glass preparations from cultures on agar only exceptionally show chains. At times, under a power of about two hundred and

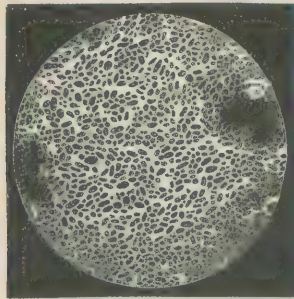
fifty diameters, short loops are observed on the margin of the colonies, that appear like streptococci. In bouillon cultures chains appear oftener.

The question repeatedly recurs to me, whether in these cultures we are not, after all, concerned with *Streptococcus pyogenes*? I still



incline to the view, however, that such is not the case. The appearance of the cultures on agar cannot be said to be typical for *Streptococcus pyogenes*. In the second place, the scarcity of chains in the cultures on agar, as well as in the pus of tooth-pulps, does not seem

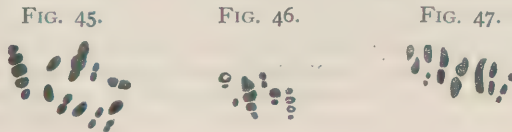
FIG. 44.



Cover-glass preparation from an older culture of the coccus seen in Fig. 36. Predominance of involution forms. After the photogram. 1000:1.

to warrant the conclusion that we have to do with a streptococcus, although, in liquid cultures, chains are very often found.

The micro-organisms of this group appear to have considerable tenacity of life. Cultures on gelatin from twelve different pulps were all found to be alive at the expiration of ten weeks.



I have, furthermore, in five cases (11, 36, 37, 42, and 45), obtained cultures of a coccus that grows in large white or gray-white colonies with a luster very much like mother of pearl; under the microscope, roundish steel-gray colonies without sharp contour. They sometimes have a scalloped border, and are brownish-yellow toward the center;

cocci and diplococci of various sizes, some of them strikingly large (Figs. 48 and 49). I did not study them more closely, as they only appeared in small numbers.

From Case 9 I obtained delicate gray colonies of large barrel-shaped cocci.

In one case, there grew isolated colonies of sarcina; in another case I found *Micrococcus tetragenus* in the blood of a mouse that had died of a mixed infection from a suppurating pulp. In two cases I observed isolated large white colonies with a yellowish center and of very tough consistency, and three times I found about a dozen large grayish-white colonies that grew well on gelatin and had a leathery consistency. Large, roundish cocci.

Furthermore, there grew in pulp 19 about one thousand rather large, grayish-blue, round colonies that appeared colorless under the microscope, or faintly yellow, with a finely granulated surface and indistinct contour. Oblong, pointed cocci, singly or in pairs (Fig. 50).

Lastly, I obtained from pulp 49, beside the usual bluish-gray colonies, some large, semi-transparent, yellowish colonies, which are



made conspicuous by their cartilaginous nature. In respect to its morphology, this bacterium also shows characteristic shapes, of which a representation is given in Fig. 51.

b. Micrococci that do not Grow on Gelatin.

The oftener I transplanted my cultures, the smaller became the number that showed no development on gelatin.

At the present time there are, among those cultures that grow on gelatin, eight that formerly were looked upon as not growing on that medium. Probably the reason for this is to be sought in a gradual adaptation of the bacteria to artificial media, as has already been observed and described concerning other bacteria.

The cultures of micrococci that up to the present have shown no disposition to grow on gelatin were obtained from pulps 2, 4, 13, 15, 18, 45, and 46. Cultures of these on agar, where the colonies are grouped closely together, appear in transmitted light on an opaque background, as if composed of innumerable small, dull glass beads; where the colonies have run together into a continuous ridge, the latter is distinctly blue; where they lie isolated, they attain a diameter of one and a half mm., appear more grayish, opaque, and lose their beady appearance.

Under the microscope the large colonies show a dense round or star-shaped nucleus of yellowish color, with a lighter, less dense, nearly homogeneous margin without sharp contour. The center of the colony is often spotted, and distinctly separated from the marginal zone. Isolated colonies sometimes take on the shape represented in Fig. 35.

The small colonies have often, under the microscope, a greenish tinge and a radiated structure. They adhere with difficulty to the cover-glass.

These cultures consequently present a striking similarity to those of the groups growing on gelatin and described above. Morphologically they show a no less striking similarity. I am consequently in doubt whether we have not to do with cultures of the same species which have been prevented, by some chance or other, from growing on the gelatin.

I have not been able to find a micro-organism which I could designate as the micrococcus of sputum-septicaemia (pneumococcus), although I have hunted for it diligently. In the last three years I have, in more than one hundred cases, infected mice with pieces of putrid and suppurating pulps in skin-pockets; in more than twenty cases I have made emulsions of such pulps in sterilized water, and injected them subcutaneously or into the abdominal cavity of mice; and in the third place, in about thirty cases I infected mice with the liquid of condensation of agar cultures, two to three days old, made directly from the pulp. In not one of these cases did the mouse die of real sputum-septicaemia. Particularly in consequence of the last-named experiments, I have been forced to exclude the pneumococcus from the list of pulp-bacteria, since an agar culture, two days old, of the pneumococcus, obtained directly from the tissues of the human body, is invariably fatal to white mice, whether injected subcutaneously or into the pleural or peritoneal cavities.

On this point my results differ from those of Schreier, who, in the *Oesterreichisch-Ungarische Vierteljahrsschrift für Zahnärzte*, 1893, Heft II, made a communication well worth reading concerning the etiology and pathogenesis of periostitis dentalis. Schreier found the *Diplococcus pneumoniae* fifteen times in twenty cases,—*i.e.*, in seventy-five per cent.,—while I did not find it once. Of course I do not mean to assert that my results alone are correct, but the significance of my many experiments on mice cannot be denied. Furthermore, the observations which led Schreier to the diagnosis of *Diplococcus pneumoniae* seem to me to speak rather against than for it. He infected two mice with a culture (second generation) in bouillon,—one of them in the pleural, the other in the peritoneal, cavity. The latter died in sixteen hours, the former remained alive.

In the blood and the scanty exudations of the abdominal cavity he found numerous round and oblong cocci in pairs and short chains; capsules were wanting. There are three points to be emphasized in these data: 1. Death with cocci in the blood speaks neither for nor against *Diplococcus pneumoniae*, since other micrococci of the mouth—for example, *Streptococcus dentinalis* Dellewie—kill mice under exactly the same symptoms. 2. Death of only one of the two mice speaks rather *against* *Diplococcus pneumoniae*. 3. Absence of capsules speaks directly against that micro-organism.

I have also often noticed the appearances in the growth of the colonies emphasized by Schreier, a white center and a very delicate transparent rim. But this appearance is characteristic neither for bacteria of the tooth-pulp nor for *Diplococcus pneumoniae*.

The facts adduced by Schreier, that cultures nineteen to twenty-three days old, kept at room temperature, developed without excep-

tion when planted upon agar, certainly does not speak for pneumococcus, if not directly against it.

Pneumococci from the blood of a mouse, planted on glycerin-agar, thrive rapidly at blood temperature; the colonies attain a considerable size in twenty-four hours, after which time they do not grow much more. After twenty-four hours they resemble dewdrops (Fränkel), or look rather more like thin starch paste,—*i.e.*, they are usually not quite transparent, but somewhat clouded. After another twenty-four hours the colonies look flatter, as if they had begun to dry out. After three days they often show a halo and a transparent nucleus.

PATHOGENESIS OF THE PULP-BACTERIA.

There are, then, as I have already pointed out, different species of bacteria in the diseased pulp that have not yet been cultivated on artificial media, and of whose pathogenesis we know nothing definite.

Their great numbers in some pulps, and especially the repeated occurrence of spirochætes, justify the supposition that, under certain circumstances, they may play an important rôle in suppurative processes. We have all the more reason for such a supposition from the fact that spirochætes and spirilla have also been found in almost pure culture in abscesses of the jaw of dental origin.

The pathogenesis of the specific pyogenic bacteria found in the pulp (*Staphylococcus pyogenes* and *Streptococcus pyogenes*) is well known, and need not be dwelt upon here. The other forms which I have isolated, and particularly the group illustrated in Figs. 33-47, demand thorough consideration.

The pathogenic action of these bacteria was tested solely on white mice by subcutaneous or intraperitoneal injections of bouillon cultures or, more commonly, of the liquid of condensation of agar cultures. In a few cases the mice were inoculated in skin-pockets.

The subcutaneous injections proved to be the most satisfactory, for even pus-producing bacteria are often resorbed by the peritoneum without exhibiting any appreciable symptoms of disease.

1. *Bacilli*.

Reference has already been made to the slight reaction following the subcutaneous injection of pure cultures of the bacilli of the pulp.

A pyogenic action was observed only on the part of the bacillus illustrated in Fig. 28. In one case an injection of a pure culture of this bacillus was followed by the formation of a small abscess with white pus. In a second case a slight diffuse suppuration was produced.

Occasionally very small pus-centers, not much larger than the head of a pin, were observed as the result of an injection of considerable quantities of other pulp-bacilli.

In mixed infections of bacilli and cocci, the former do not, however, necessarily die. On the contrary, they sometimes seem to develop further, so that a certain pathogenic action on their part cannot be denied where they act together with cocci.

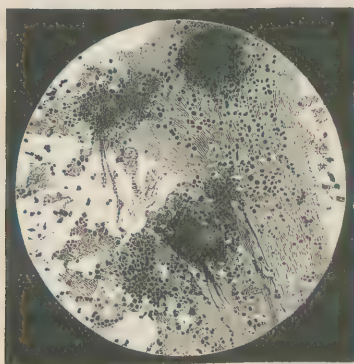
Three cases in which bacilli were found in the pulp, which caused death by blood-poisoning, have been described in detail above.

2. *Micrococci.*

The cultures of the micrococci of the pulp proved to be much more virulent than those of the bacilli.

The reaction did not, it is true, occur invariably, but in the majority of the subcutaneous injections in mice pus-centers from the size of a barleycorn to that of a pea were formed, in which the cocci could easily be seen under the microscope and obtained again in pure culture. Occasionally bean-sized pus-centers appeared, in others the suppuration was more diffuse. It occurred with sufficient constancy to prove that we here had to do with specifically pus-producing micro-organisms. For even the staphylococci and streptococci do not invariably produce suppuration; on the contrary, the injections sometimes disappear entirely without causing the least disturbance. In a number of cases where a single large colony was taken from a plate of agar and introduced into a skin-pocket, a very similar result was produced.

FIG. 52.



Cover-glass preparation from the pus of an abscess produced by a mixed infection from a diseased pulp. After the photogram. 1000:1.

If we vaccinate a mouse subcutaneously with a mixed culture from a putrid pulp, the operation will be followed by the formation of an abscess in the pus of which we find chiefly, or only, micrococci (Fig. 52). This result points to the conclusion, which we had already arrived at by other means, that the power to produce suppuration in the dental pulp adheres chiefly to the micrococci and not to the bacilli. There is, however, but little doubt that the infectious nature of the putrid pulp is intensified by the presence of the bacilli and the products which they may help to form.

EFFECT OF INCORPORATING PORTIONS OF PUTRID PULPS.

Bacteriological investigation has shown that large masses of living bacteria are by no means always present in putrid pulps. In fact, it frequently happens that in cultures from such pulps no development whatever takes place. This fact led Schreier to the conclusion that a putrid pulp in such cases must be a very harmless thing,—a view which is not supported by the results of experiments or by clinical experience. The virulence of putrid pulp-tissue does not depend

alone upon the numbers of bacteria, but in a high degree also upon the products of putrefaction (ptomaines) present. I long ago called

FIG. 53.



Agar tube containing a piece of putrid pulp-tissue at *a*. Disruption of the agar by gas.

attention to the fact that portions of putrid pulps always act more intensely than portions of inflamed or suppurating pulps, and more intensely than pure cultures of bacteria from such pulps. These experimental results are readily confirmed by clinical experience, which has taught us that of all conditions of the diseased pulp none is so readily followed by evil consequences as that in which the pulp is converted into a stinking putrid mass.

We have every reason to suppose that putrid pulps, even when free from bacteria,—*i.e.*, in which the bacteria have died for lack of nourishment, or from the action of their own poisonous products,—may produce inflammation and suppuration which, only if a single living germ be present, may assume a progressive character.

A putrid pulp which is absolutely free from living organisms is, however, probably never met with; but since, granted that the bacteria of the main body of the pulp may have died from lack of nutrition, those at the apical foramen might well be able to sustain life much longer by reason of the fact that a slight infiltration of the tissue of the pulp at the foramen must take place from the liquids of the surrounding tissue.

In abscesses produced by mixed inoculations with portions of putrid pulps, or with emulsions from such pulps, we regularly find a preponderance of micrococci both under the microscope and in the cultures.

PUTREFACTIVE PROCESSES IN THE DENTAL PULP.

We are all perfectly well acquainted with the fact that surprising quantities of foul-smelling products may be found in the decomposition of the dental pulp. This circumstance, however, admits of a ready explanation. When a small amount of organic matter, such as the pulp of the human tooth, putrefies in open air, the products of decomposition in a great measure become oxidized or are carried away by the movements of the air. In a closed pulp-chamber neither of these factors is in operation, so that gases and other bad-smelling products of putrefaction may accumulate in comparatively large quantities.

The odors developed by the pulp are, as is well known, of different kinds and vary greatly in intensity. They are determined on the one hand by the nature of the bacterium or bacteria present, and on the other by the stage of putrefaction. If the pulp-chamber is open so as to admit

particles of food, particularly carbohydrates, we will have a mixture of putrid and sour smells. Where the apical foramen is large, and particularly where the wall of the root-canal has been broken through, the odor will be further modified by the decomposition of the ingressing serum.

Among the products of decomposition I have easily found ammonia and sulfuretted hydrogen. Undoubtedly a series of other products are present, of which we should expect in first line sulfid of ammonia, phosphoretted hydrogen, indol, skatol, etc.

If we bring a putrid pulp into a sterilized test-tube and fill over it with glycerin-agar, we shall observe in the following days a more or less marked development of gas by which the agar will usually be torn to pieces, as shown in Fig. 53.

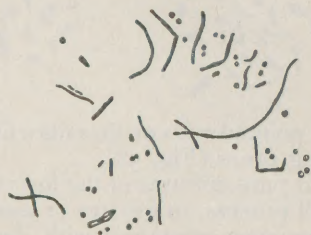
If we hang a piece of moist lead-paper in such a tube, it will, if left for some time, turn black (H_2S), while a moistened strip of red litmus-paper will often turn blue (NH_3).

If we open the tube, we have no difficulty in convincing ourselves of the intensity of the putrefactive process present. The contents of such a tube, in particular the liquid which usually accumulates on the bottom, are very infectious.

FIG. 54.



FIG. 55.



In order to determine what action the access or exclusion of air may have on the virulence of the products of putrefaction, the following experiments were made: A few drops of gelatin were placed in a test-tube, infected with a portion of a putrid pulp, and hardened in cold water. This was then covered with a column of agar; at first only a drop or two at a time was added, the tube being kept in cold water so as not to melt the gelatin.

In the control experiments the agar was first placed in the tube and a few drops of gelatin placed on top of it; this was then infected with a piece of the same pulp. In one case the gelatin was protected from the air, in the other the air had free access. Subcutaneous infections with cultures obtained in this way produced almost unexceptionally stronger reaction (more extensive suppuration) where the culture had developed under free access of air.

These results render it probable that, although bacteria may grow where the amount of oxygen present is exceedingly limited, as in a closed pulp-chamber, they develop better and attain greater virulence under access of air.

The question whether there is a specific bacterium of the putrid pulp was also made the subject of a few experiments. In Figs. 17-23 we have already made the acquaintance of the various forms of bac-

teria found in the putrid pulp. In Figs. 54-58 we have also the forms which were obtained when portions of putrid pulps were kept for a number of days in a column of agar at the temperature of $35-37^{\circ}\text{C}$.

We find,—

1. As a constant appearance small cocci and diplococci, very like those which we have established as the pyogenic cocci of the pulp (Figs. 54-57).

2. Somewhat less regularly slender, straight, or curved rods of various lengths which sometimes grow out into long threads (Figs. 54-55).

3. We often find also short rods with round ends, which are characterized by their bipolar staining.

In Fig. 56 we have a culture from a putrid pulp, showing the large involution forms which we have examined into above. (Compare Figs. 36-47.)

4. A species of micrococcus, which shows a great resemblance to *Micrococcus tenuis*, is occasionally met with (Fig. 57).

FIG. 56.



FIG. 57.



FIG. 58.



5. Long, pointed rods or threads which take up the coloring-matter in alternating zones (Fig. 58).

I obtained pure cultures of the forms referred to under 1, 2, 3, and

4. They all possess, in greater or less degree, the power of decomposing albuminous substances with development of putrid products. The chief rôle seems to be performed by the coccus described under 1. Further experiments are, however, necessary to furnish a definite solution of this question.

In regard to the last question placed at the beginning of this communication, Have we remedies at our disposal which are capable of rendering putrid pulps innocuous for the animal organisms? my experiments are not far enough advanced to justify me in presenting the results to the profession. This I hope to be able to do in a future paper.

